REMARKS

Applicant respectfully requests reconsideration of the above-identified patent application in view of the amendment above and the remarks below.

No claims have been canceled or added herein. Claims 1, 12 and 14 have been amended herein. Therefore, claims 1-14 are pending and are under active consideration.

Claims 1-3, 6-10, 12 and 13 stand rejected under 35 USC 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000, filed January 5, 1998)." In support of the rejection, the Patent Office states the following:

> Applicants' arguments received on November 24, 2004 have been fully considered but they are not found persuasive for the following reasons.
>
> Applicants' arguments are addressed in the same order they were received.
>
> ...
>
> Applicants also contend that Gonzalgo et al. not only employ radioactive markers for their detection instead of fluorescently labeled markers, but the radioactive marker is incorporated after the amplification of the genomic DNA has already been performed.
>
> This argument is not found persuasive because had Gonzalgo et al. taught all of the claimed limitations as stated by Applicants, such art would have been rejected under a different statu[t]e, namely under 35 U.S.C. 102(b).
>
> The teachings of Gonzalgo et al. relied upon is the steps of:
>
> (a) treating a genomic DNA sample with bisulfite to convert non-5'-methylated cytosines to uracils while not converting the 5' methylated cytosines; and

(b) amplification of the resulting genomic DNA sample.

Gonzalgo et al. analyzes and separates the amplified product via electrophoresis (page 5, line 24).

Gonzalgo et al., however, in the detection of the CpG, employs a methylation-sensitive single nucleotide primer extension (Abstract).

Yurov et al. and Davis et al. disclose a well-known method of amplifying and detecting target nucleic acid sequences via use of fluorescently labeled nucleotides, Cy3 and/or Cy5 (column 19, lines 18-20, Davis et al.) as well as the advantage of using Cy3 or Cy5 labels rather than other fluorescent labels:

"Cyanine dyes are also useful as fluorescent labels or biological macromolecules. Cyanine 3 dye provides **significantly brighter fluorescence** than any other fluorophore, including fluorescein..." (page 391, 1st column, Yurov et al.)

The use of fluorescently labeled nucleotides in generating target amplicons is well-known and practiced in the art of nucleic acid detections. Such teachings are prevalent, such as Affymetrix GeneChip™ which amplifies target nucleic acid sequences via use of fluorescently labeled nucleotides, followed by their hybridization to an array of immobilized probes.

Therefore, one of ordinary skill in the art at the time the invention was made would have been reasonably motivated to modify the teachings of Gonzalgo et al. to employ the fluorescent labeled nucleotides for the detection of bisulfite treated genomic DNA.

Applicant respectfully traverses the subject rejection. Claims 2-3, 6-10, 12 and 13 depend from claim 1. Claim 1, which has been amended herein, now recites "[a] method for the relative quantification of the methylation of cytosine bases in DNA samples, said method comprising the steps of:

a) chemically reacting a genomic DNA sample with a reagent, wherein 5-methylcytosine and cytosine react differently and these thus show a different base pairing behavior in the DNA duplex after the reaction;

b) then, amplifying the chemically reacted DNA sample, said amplifying step comprising the use of a fluorescently labeled dCTP or dGTP derivative to yield amplified products;

c) then, spatially separating the amplified products from each other;

d) then, quantitatively measuring the fluorescence of the separated amplified products; and

e) then, determining from the measured fluorescence the relative number of methylated cytosine bases that were present in the DNA sample prior to step a)."

Claim 1 is patentable over the applied references for at least the reason that the applied references, whether taken individually or in combination, do not teach or suggest a method for determining the **relative number** of methylated cytosine bases in a DNA sample.

Gonzalgo et al. does not relate to a method for determining the **relative number** of methylated cytosine bases in a DNA sample, but rather, is directed at a method for determining whether or not a cytosine **at a particular location** is methylated. More specifically, the Gonzalgo method involves (a) obtaining genomic DNA from a DNA sample to be assayed; (b) reacting the genomic DNA with sodium bisulfite to convert unmethylated cytosine residues to uracil residues while leaving any 5-methylcytosine residues unchanged to provide primers specific for the bisulfite-converted genomic sample for top strand or bottom strand methylation analysis; (c) performing a PCR amplification procedure using the top strand or bottom strand specific primers; (d) isolating the PCR amplification products; (e) performing a primer extension reaction using Ms-SNuPE primers, [^{32}P]dNTPs and *Taq* polymerase, wherein the Ms-SNuPE primers comprise a from about a 15 mer to

about a 22 mer length primer that terminates immediately 5' of a single nucleotide to be assayed; and (f) determining the relative amount of allelic expression of CpG methylated sites by measuring the incorporation of different ^{32}P-labeled dNTPs.

As can be seen from the above, in order to provide an appropriate 15mer to 22mer primer that terminates immediately 5' of the single nucleotide to be assayed, the Gonzalgo method requires one to have previous knowledge of the sequence context in which the cytosine in question occurs. By contrast, the claimed method does not require any prior sequence knowledge as it is only the **relative number** of cytosine methylations, and **not the locations** of the cytosine methylations, that is determined. In other words, the Gonzalgo method and the claimed method provide different pieces of information about a sequence, the Gonzalgo method indicating the methylation status of a cytosine **at a particular location**, the claimed method indicating the **relative number** of methylated cytosines present in a sequence. Applications for the type of information provided by the claimed method, as contrasted with that of the Gonzalgo method, include determining the presence and extent of "co-methylation," a biological phenomenon in which a majority of CpG positions within a CpG rich region share the same methylation status, and detecting and/or observing progressive co-methylation within, for example, aging, differentiating or diseased tissue. The Gonzalgo method is unsuited for such applications as each primer extension reaction can only be used to detect the methylation status at an individual position; consequently, a different assay needs to be performed for the analysis of each CpG position in accordance with Gonzalgo et al. On the other hand, the Gonzalgo method is well-suited in diagnostic or clinical settings where a high degree of accuracy is needed with respect to a small number of CpG positions.

Yurov et al. and Davis et al., which are relied upon by the Patent Office for allegedly teaching fluorescently-labeled nucleotides, do not relate to a method for determining the relative number of cytosine methylations in a sample and, therefore, do not cure the above-noted deficiencies of Gonzalgo et al. Moreover, there is no basis for modifying Gonzalgo et al. to eliminate its primer extension step and, instead, to perform a PCR amplification using the fluorescent markers of Yurov et al. and Davis et al. It is well-settled that there must be some teaching or suggestion in the prior art to modify the prior art so as to arrive at the claimed invention. Applicant respectfully submits that the Patent Office has failed to carry its burden of proving that its proposed modifications are taught or suggested by the prior art. The Patent Office cannot merely pick and choose elements from the prior art and combine them in any conceivable fashion. Applicant has noted previously that there is no teaching or suggestion in the prior art of the desirability of eliminating the primer extension step of Gonzalgo et al. In fact, as noted by Applicant, such a modification would constitute a complete overhaul and destruction of the Gonzalgo method and would be contrary to the basic principle behind the Gonzalgo method - factors that are highly probative against making the proposed modification. The Patent Office cannot simply dismiss such deficiencies by stating the truism that "had Gonzalgo et al. taught all of the claimed limitations as stated by Applicants, such art would have been rejected under a different statu[t]e, namely under 35 U.S.C. 102(b)" as such a rationalization could be applied to any reference used in a 103 rejection. Instead, the Patent Office must explain why a person of ordinary skill in the art would have been motivated to modify the Gonzalgo method by eliminating the primer extension step, using fluorescently labeled dCTP or dGTP in the claimed amplification step, and making the other modifications necessary to arrive at

the claimed invention. Applicant respectfully submits that the Patent Office has failed to meet its burden.

Therefore, for at least the above reasons, claim 1 is patentable over Gonzalgo et al. in view of Yurov et al. and in light of Davis et al.

Claims 2-3, 6-10, 12 and 13, which depend from claim 1 and recite additional features, are further patentable over Gonzalgo et al. in view of Yurov et al. and in light of Davis et al. for at least the reasons of record.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 4 and 5 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000, filed January 5, 1998), as applied to claims 1-3, 6-10, 12, and 13 above, and further in view of Apffel et al. (U.S. Patent No. 6,379,889 B1, issued April 30, 2002, filed November 4, 1999) and Roche et al. (Biotechnology Progress, 1997, vol. 13, pages 659-668)." In support of the rejection, the Patent Office states the following:

> Claims are drawn to a method of quantitating the methylation of cytosine bases in DNA sample wherein the separation of the PCR products is achieved by either High Performance Liquid Chromatography (HPLC) or Capillary Gel Electrophoresis (CGE).
>
> The teachings of Gonzalgo et al. Yurov et al. and Davis et al. have already been discussed above.
>
> Gonzalgo et al., Yurov et al. and Davis et al. do not explicitly disclose the use of HPLC or CGE for PCR product separation.
>
> Apfell et al. disclose a method of using HPLC for the separation of PCR amplicons from a PCR reaction mixture (column 3, lines 45-48)

Roche et al. disclose a method of using GCE for the separation of PCR amplicons (pp. 663, 2nd column bottom).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to expand the teachings of Gonzalgo et al., Yurov et al. and Davis et al. with the teachings of Apffel et al. and Roche et al. to arrive at the invention as claimed per suggestion offered by Gonzalgo et al., wherein the artisans state:

"There are many chromatographic techniques that can be used to isolate PCR amplification products (*or amplicons*)" (pp. 8, line 7-9).

One of ordinary skill in the art at the time the invention was made would have recognized various chromatographic techniques for separation/purification and the advantage offered by such techniques, as illustrated by Apffel et al. and Roche et al.:

"CE is capable of rapid, automated, reproducible, and high-resolution separation of small volumes of complex mixtures." (pp. 659, 2nd column; pp. 664, 1st column, *Roche*).

"Distinguish individual PCR amplicons (also referred to as PCR products herein) from a PCR reaction mixture." (column 3, lines 44-47).

Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to modify the teachings of Gonzalgo et al., Yurov et al. and Davis et al. given their explicit statement of feasibility to realize the advantages offered by the separation teachniques of Apffel et al. and Roche et al. with a reasonable expectation of success.

Therefore, the invention as claimed is obvious over the cited references.

Applicant respectfully traverses the subject rejection. Claims 4 and 5 depend from claim 1. Claim 1 is patentable over Gonzalgo et al. in view of Yurov et al. and in light of Davis et al. for at least the reasons given above. Apffel et al. and Roche et al. do not cure all of the deficiencies of

Gonzalgo et al., Yurov et al. and Davis et al. Therefore, based at least on their respective dependencies from claim1, claims 4 and 5 are patentable over the applied references.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 11 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000, filed January 5, 1998), as applied to claim 1 above, and further in view of Wang et al. (Science, May 1998, vol. 280, pages 1077-1082)" In support of the rejection, the Patent Office states the following:

> The teachings of Gonzalgo et al., Yurov et al., and Davis et al. have been set forth above.
>
> Gonzalgo et al., Yurov et al., and Davis et al. do not explicitly disclose that the amplification was multiplexed.
>
> Wang et al. disclose a method of SNP genotyping which involves multiplex amplification from a genomic DNA via plurality of primers (pp. 1080). Wang et al. multiplexes 46 loci from a genomic DNA (pp. 1080, 3rd column).
>
> It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Gonzalgo et al., Yurov et al., and Davis et al. with the teachings and advantages disclosed by Wang et al. to arrive at the invention as claimed for the following reason.
>
> Wang et al. clearly suggest the well-known advantage of multiplexing PCR reactions:
>
> We next sought to decrease substantially the sample preparation required to genotype large numbers of SNPs, as required to perform a genomic scan. We developed a protocol based on multiplex PCR in which primer pairs from many different loci are combined in a single reaction." (page 1080, 3rd column).
>
> One of ordinary skill in the art, therefore, would have been motivated to employ the well-known multiplex-PCR technique into

> the method disclosed by Gonzalgo et al., Yurov et al., and Davis et al. for the well-known advantage of reducing the sample preparation/contamination with a reasonable expectation of success.
>
> Therefore, the invention as claimed is obvious over the cited references.

Applicant respectfully traverses the subject rejection. Claim 11 depends from claim 1. Claim 1 is patentable over Gonzalgo et al. in view of Yurov et al. and in light of Davis et al. for at least the reasons given above. Wang et al. does not cure all of the deficiencies of Gonzalgo et al., Yurov et al. and Davis et al. Therefore, based at least on its dependency from claim 1, claim 11 is patentable over the applied references.

Claim 14 stands rejected under Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000, filed January 5, 1998)." In support of the rejection, the Patent Office repeats the same arguments presented above in connection with the rejection of claims 1-3, 6-10, 12 and 13 and then concludes as follows:

> With regard to the method being "consisting of" the recited steps (a) through (d), the invention as claimed is determined to be obvious as one of ordinary skill in the art at the time the invention was made, when combined with the teachings of Gonzalgo et al., Yurov et al., and Davis et al. would have arrived at the method of the recited steps.

Applicant respectfully traverses the subject rejection for at least the same reasons given above in connection with the rejection of claims 1-3, 6-10, 12 and 13. With respect to the Patent Office's comments regarding the transitional phrase "consisting of," Applicant respectfully submits that the Patent Office has failed to give any weight to the fact that "consisting of" is used instead of "comprising." It is not sufficient for the Patent Office simply to conclude that "the invention as

claimed is determined to be obvious as one of ordinary skill in the art....would have arrived at the method of the recited steps." Instead, the Patent Office must explain why one of ordinary skill in the art would have been motivated to make all of the modifications needed to change the Gonzalgo method to the claimed method, including eliminating the primer extension step. Applicant respectfully submits that the Patent Office has failed to provide such an explanation.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-13 stand provisionally rejected "under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-29 of copending Application No. 10/220,090...for the reasons of record."

Applicant respectfully traverses the subject rejection. Claim 1 of USSN 10/220,090, from which claims 2-29 depend of USSN 10/220,090 depend, recites "[a] method for detecting 5-methylcytosine in genomic DNA samples, characterized in that the following steps are carried out:

(a) a genomic DNA from a DNA sample is chemically converted with a reagent, 5-methylcytosine and cytosine reacting differently, thus exhibiting different base pairing behaviors in the DNA duplex subsequent to the reaction;

(b) the pretreated DNA is amplified using a polymerase and at least one oligonucleotide (type A) as a primer;

(c) the amplified genomic DNA is hybridized to at least one oligonucleotide (type B), forming a duplex, said hybridized oligonucleotides of type B, with their 3'-ends, immediately or at a distance of up to 10 bases, adjoining the positions to be analyzed with regard to their methylation in the genomic DNA sample;

(d) the oligonucleotide (type B) having a known sequence of n nucleotides is elongated by means of a polymerase by a plurality of nucleotides, at least one nucleotide carrying a detectable label, and the elongation depending on the methylation status of the specific cytosine in the genomic DNA sample;

(e) the elongated oligonucleotides are analyzed for the presence of the label."

Applicant respectfully submits that claims 1-13 of the present application are patentably distinguishable over claims 1-29 of USSN 10/220,090 for at least the reasons that (i) claims 1-29 of USSN 10/220,090 do not teach or suggest, among other things, claim 1, step (e), of the present application, namely, determining from the measured fluorescence the relative number of methylated cytosine bases that were present in the DNA sample prior to step a); and (ii) claims 1-13 of the present application do not teach or suggest, among other things, steps (c) and (d) of claim 1 of USSN 10/220,090.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-13 stand provisionally rejected "under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-26 of copending Application No. 10/220,896...for the reasons of record."

Applicant respectfully traverses the subject rejection for the same types of reasons given above in connection with the provisional double patenting rejection based on USSN 10/220,090.

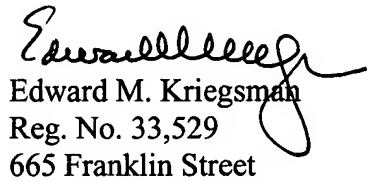
In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

By: 

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on August 22, 2005

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